CLAIMS

In the claims:

1. A method of treating critical limb ischemia (CLI) comprising administering to a patient in need of treatment an effective amount of a polynucleotide encoding a mammalian eNOS polypeptide.

2. The method according to Claim 1, wherein said eNOS polypeptide is a human eNOS polypeptide.

3. The method according to Claim 2, wherein the amino acid sequence of said human eNOS polypeptide is SEQ ID NO: 1.

4. The method according to Claim 3, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in said human eNOS that is phosphorylated in mammalian cells.

5. The method according to Claim 4, wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid residue 495 of SEQ ID NO: 1.

6. The method according to Claim 4, wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1.

7. The method according to Claim 4, wherein said eNOS polypeptide comprises a first mutation at a position corresponding to amino acid 495 and a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1.

8. The method according to Claim 4, wherein said eNOS polypeptide comprises a first mutation at a position corresponding to amino acid 495, a second mutation at a position corresponding to amino acid 1177, and a third mutation at a position corresponding to amino acid 2 of SEQ ID NO: 1.

9. The method according to Claim 6, 7, or 8, wherein said mutation at a position corresponding to amino acid residue 495 is an amino acid substitution to Ala, Val, Leu, or Ile.

10. The method according to Claim 6, 7, or 8, wherein said mutation at a position corresponding to amino acid residue 1177 is an amino acid substitution to Asp.

11. The method according to Claim 8, wherein said mutation at a position corresponding to amino acid residue 2 is an amino acid substitution to Ala.

12. The method according to Claim 4, wherein the phosphorylation of said eNOS polypeptide is increased or decreased, as compared to a reference eNOS polypeptide.

13. The method according to Claim 4, wherein said eNOS polypeptide has an increased binding affinity for calmodulin, as compared to a reference eNOS polypeptide.

14. The method according to Claim 4, wherein Ca++ dependence is decreased in Ca++-calmodulin mediated stimulation of said eNOS polypeptide as compared to a reference eNOS polypeptide.

15. The method according to Claim 4, wherein said eNOS polypeptide has increased eNOS activity, as compared to a reference eNOS polypeptide.

16. The method according to Claim 15, wherein said activity is the generation of NO.

17. The method according to Claim 15, wherein said activity is reductase activity.

18. The method according to Claim 12, 13, 14, 15, 16, or 17, wherein the amino acid sequence of said reference polypeptide is, or is derived from, the amino acid sequence of a human eNOS.

19. The method according to Claim 18, wherein the amino acid sequence of said reference polypeptide is, or is derived from, SEQ ID NO: 1.

20. The method according to Claim 4, wherein the amino acid sequence of said eNOS polypeptide is substantially homologous to the amino acid sequence of a human eNOS.

21. The method according to Claim 20, wherein the amino acid sequence of said eNOS polypeptide has a 95-99 % sequence identity to the amino acid sequence of SEQ ID NO: 1.

22. The method according to Claim 1 or 4, wherein said polynucleotide is a recombinant vector comprising a nucleic acid sequence encoding said eNOS polypeptide and said sequence is operably linked to at least one regulatory sequence such that said polypeptide is expressed in cells.

23. The method according to Claim 22, wherein said nucleic acid sequence is operably linked to a promoter.

24. The method according to Claim 23, wherein said recombinant vector is a viral vector.

25. The method according to Claim 24, wherein said viral vector is an adenoviral vector.

26. The method according to Claim 1 or 4, wherein said treating comprises modulating eNOS activity in cells of said patient.

27. The method according to Claim 26, wherein said cells are endothelial cells.

28. The method according to Claim 26, wherein said cells are bone marrow derived cells.

29. The method according to Claim 1 or 4, wherein said method further comprises administering one or more angiogenic factors to said patient, before, during, or after said administering of said polynucleotide.

30. The method according to Claim 29, wherein said angiogenic factors are selected from a group of angiogenic factors consisting of: HGF, VEGF, FGF, Endothelial Growth Factor, Epidermal Growth Factor, Platelet-Derived Growth Factor, TGF-alpha, TGF-beta, PDGF, TNA-alpha or IGF, Del-1.

31. The method according to Claim 1 or 4, wherein said administering comprises introducing said polynucleotide to cells of said patient ex vivo.

32. The method according to Claim 1 or 4, wherein said administering comprises delivery of said polynucleotide to a diseased tissue of said patient.

33. The method according to Claim 1 or 4, wherein said administering comprises delivery of said polynucleotide to the peripheral vascular system of said patient.

34. The method according to Claim 33, wherein said delivery is by intramuscular injection or intraarterial injection to a limb muscle of said patient.

35. A method of treating angiogenesis comprising administering to a patient in need of treatment an effective amount of a polynucleotide encoding an eNOS polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a mammalian eNOS that is phosphorylated in mammalian cells.

36. A method of ameliorating microvascular dysfunction comprising administering to a patient in need of treatment an effective amount of a polynucleotide encoding an eNOS polypeptide, wherein said

eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a mammalian eNOS that is phosphorylated in mammalian cells.

37. A method of treating critical limb ischemia (CLI) comprising administering to a patient in need of treatment an effective amount of an eNOS polypeptide, wherein said eNOS polypeptide comprises at least one mutation at a position corresponding to an amino acid residue in a mammalian eNOS that is phosphorylated in mammalian cells.

38. The method according to Claim 35, 36, or 37, wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid residue 495 of SEQ ID NO: 1, and said mutation is an amino acid substitution to Ala, Val, Leu, or Ile.

39. The method according to Claim 35, 36, or 37, wherein said eNOS polypeptide comprises a mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said mutation is an amino acid substitution to Asp.

40. The method according to Claim 1, 35, 36, or 37, wherein said eNOS polypeptide comprises:

i) a first mutation at a position corresponding to amino acid 495, and said first mutation is an amino acid substitution to Ala, Val, Leu, or Ile; and

ii) a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Asp.

41. The method according to Claim 1, 35, 36, or 37, wherein said eNOS polypeptide comprises:

i) a first mutation at a position corresponding to amino acid 495, and said first mutation is an amino acid substitution to Ala, Val, Leu, or Ile;

ii) a second mutation at a position corresponding to amino acid 1177 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Asp; and

iii) a third mutation at a position corresponding to amino acid 2 of SEQ ID NO: 1, and said second mutation is an amino acid substitution to Ala.